CHAPTER 4

ADENOSINE DRY POWDER INHALATION FOR BRONCHIAL CHALLENGE TESTING, PART 2: PROOF OF CONCEPT IN ASTHMATIC SUBJECTS

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European Journal of Pharmaceutics and Biopharmaceutics 2014, 88(1):148-152

ABSTRACT

Adenosine is an indirect stimulus to assess bronchial hyperresponsiveness (BHR) in asthma. Bronchial challenge tests are usually performed with nebulised solutions of adenosine 5'-monophosphate (AMP). The nebulised AMP test has several disadvantages, like long administration times and a restrictive maximum concentration that does not result in BHR in all patients. In this study, we investigated the applicability of dry powder adenosine for assessment of BHR in comparison to nebulised AMP. Dry powder adenosine was prepared in doubling doses (0.01–80 mg) derived from the nebulised AMP test with addition of two higher doses. Five asthmatic subjects performed two bronchial challenge tests, one with nebulised AMP following the 2-minute tidal breathing method; the second with dry powder adenosine administered with an investigational inhaler and single slow inhalations (inspiratory flow rate 30–40 L/min). All subjects reached a 20% fall in FEV_1 with the new adenosine test (PD_{20}) compared to four subjects with the AMP test (PC_{20}). Dry powder adenosine was well tolerated by all subjects and better appreciated than nebulised AMP. In conclusion, this new bronchial challenge test appears to be a safe and convenient alternative to the nebulised AMP test to assess BHR in asthmatic subjects.

INTRODUCTION

Bronchial challenge tests are used to measure bronchial hyperresponsiveness (BHR), a hallmark of asthma. These tests are usually performed with methacholine, which acts directly on the airway smooth muscle cells (36). However, it has been described that BHR in response to indirectly acting stimuli, such as adenosine, may better reflect bronchial inflammation than BHR to methacholine (43,44).

Recently, it was investigated whether bronchial challenge testing with small and large particles aerosolised adenosine 5'-monophosphate (AMP) can discriminate between asthmatic subjects that respond well to treatment with either small or large particles inhaled corticosteroids (ICS) (55). A significant improvement was observed in the provocative concentration causing a 20% fall in FEV_1 (PC_{20}) with the small particles AMP in subjects receiving small particles ICS, whereas there was no improvement in subjects receiving large particles ICS. We therefore believe that the concept of identifying asthmatics with small airways dysfunction by challenging them with small particle AMP is valid. However, because the bronchial challenge with small particles AMP led to a 20% fall in FEV_1 in only 60% of the subjects, we clearly need to further optimise the technical (administration-related) aspects of this test.

Although adenosine is the agent that ultimately leads to smooth muscle constriction, solutions of AMP are being used because of its higher solubility compared with adenosine. Historically, BHR challenge tests have mostly been performed with provoking agents diluted in 0.9% saline that are administered by nebulisation. Doubling concentrations of AMP up to a maximum of 300–400 mg/mL are administered, following methacholine bronchial challenge test protocols (225). Such high AMP concentrations have been shown to greatly affect nebuliser performance in terms of droplet size and output rate, leading to smaller (but relatively heavier) droplets and a lower output rate at higher concentrations (**Chapter 2**). The differences in droplet size imply that the site of deposition differs between low and high AMP concentrations, whereas the effect on output rate results in differences in administered volume (dose). Therefore, differences in response to low and high concentrations cannot be assigned to concentration alone.

Another disadvantage of the current test is that tidal breathing with small volumes (±500 mL) is not a very effective method to wash-in relatively large functional residual capacity (FRC) volumes (±2000 mL), resulting in long administration times, which are burdensome to both the patient and the lung function lab. In addition, the

use of solutions of the stimulus leads to stability concerns upon storage (243).

All of the issues described above can be addressed by replacement of the nebulisation procedure by dry powder inhalation. With a dry powder inhaler (DPI), adenosine can be used instead of its precursor AMP as solubility is no longer an issue. Adenosine is stable in the dry state, so DPI formulations can be stored for relatively long periods of time. Additionally, a DPI enables the administration of a dose in one single inhalation, which significantly reduces the administration time. Moreover, by using an effective inhaler, the particle size is independent of the drug dose leading to similar deposition for both low and high doses. Consequently, regional targeting can be facilitated using different particle sizes for the stimulus and controlling the flow rate at which the particles are administered (22).

We have developed a dry powder test concept for bronchial challenge testing with adenosine. The development and *in vitro* performance of this test have been described in the first paper of this series (**Chapter 3**). The test consists of doubling dose steps of adenosine starting from 0.01 mg, and we could show that the fraction of the dose delivered and the particle size distribution of the aerosol are both independent of the dose (**Table 3-5, Figure 3-5**). In this pilot study, we aimed to investigate whether this new dry powder adenosine bronchial challenge test can induce a 20% fall in FEV_1 in subjects with asthma that is comparable to the fall induced with the nebulised AMP test. Secondarily, we wanted to investigate the applicability of this new test concept, with respect to both its safety and patient comfort.

SUBJECTS AND METHODS

Subjects

Five subjects with a doctor's diagnosis of asthma, 18–65 years, were included in this pilot study. The study protocol was approved by the local Medical Ethics Committee (METc number 2012.057, University Medical Center Groningen, The Netherlands) and written informed consent was obtained from all participants.

Study design

The participating subjects attended the clinic on two days with an interval of one

month maximally. On each day, a bronchial challenge test using either nebulised AMP or dry powder adenosine was performed. Subjects had to withhold their bronchodilating medication (short-acting β_2-agonists for 6 hours, ICS and long-acting β_2-agonists for 12 hours).

Adenosine dry powder

The novel adenosine dry powder test that was investigated in this study consisted of doubling dose steps in a range of 0.01–80 mg. In order to cover the entire expected dose range, three powder formulations were prepared by spray drying, which consisted of either pure (100%) adenosine, or adenosine and lactose as diluent (1% and 10% adenosine) (**Chapter 3**). Adenosine and lactose (both *Ph.Eur.* quality) were obtained from BUFA Spruyt Hillen (IJsselstein, The Netherlands). Spray drying was performed in the hospital pharmacy under Good Manufacturing Practice conditions using a Büchi B290 Mini Spray Drier (Büchi Labortechnik, Switzerland).

The doses were provided in individually sealed aluminium blisters. The 40 mg and 80 mg doses consisted of respectively two and four blisters each containing 20 mg adenosine. The dose range was derived from the regular AMP test, with addition of two higher dose steps (Table 4-1). The powder was administered using an investigational inhaler especially designed for the dispersion of the adenosine powder formulations used in this study (Figure 4-1). Its dispersion principle is based on air classifier technology (235), which is also present in the Novolizer (MEDA) and Twincer (232) DPIs. The dry powder adenosine aerosol had a mass median aerodynamic diameter

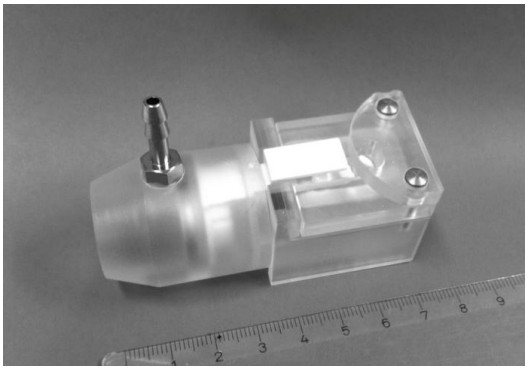

Figure 4-1. The investigational inhaler used to administer dry powder adenosine. The outlet on the mouthpiece is used for measuring the pressure drop across the inhaler during inhalation, from which the inspiratory flow rate is calculated.

Table 4-1. Conversion of AMP to adenosine.

AMP		Adenosine
Concentration (mg/mL)	Dose (mg)	Dose (mg)
0.04	0.004	-
0.08	0.007	-
0.16	0.014	0.01
0.32	0.028	0.02
0.64	0.057	0.04
1.25	0.11	0.08
2.5	0.22	0.16
5	0.44	0.32
10	0.88	0.64
20	1.8	1.25
40	3.5	2.5
80	7.1	5
160	14	10
320	28	20
-	-	40
-	-	80

The conversion is based on the estimated delivered doses of AMP, which were calculated by multiplying the AMP concentrations by the calibrated nebuliser output rate (0.13 mL/min), nebulisation time (2 min) and a duty cycle of 0.34 (**Chapter 3**).

(MMAD) of 2.6–2.9 μm and a geometric standard deviation (GSD) of 1.6 over the entire dose range when dispersed with the investigational inhaler.

Bronchial challenge test with nebulised AMP

The AMP challenge test was performed using the two-minute tidal breathing method (26,35). After a safety step with nebulised 0.9% saline, subsequent doubling AMP concentrations of 0.04–320 mg/mL were inhaled for 2 min, followed by a 3 min interval between the nebulisation steps. Ninety seconds after every step, an forced vital capacity (FVC) manoeuvre was performed, obtaining the FEV_1, FVC, and forced expiratory flow at 50% of the forced vital capacity ($FEF_{50\%}$).

Bronchial challenge test with dry powder adenosine

The adenosine challenge test was performed by inhalation of subsequent doubling adenosine doses of 0.01–80 mg with a single slow inspiratory manoeuvre, followed by a 3 min interval between the inhalations (the 40 and 80 mg doses were administered using two and four inhalations respectively). The subjects were instructed to exhale completely, to subsequently inhale as long as possible at a flow rate of 30–40 L/min, and finally to hold their breath for 10 s at maximal inspiration. To control and record the inspiratory flow rate during inhalation, the inhaler was connected to a flow measurement device with a visual feedback system, showing the actual flow rate of the subject's inhalation on a computer screen. The 40 mg and 80 mg doses were respectively inhaled in two and four consecutive inhalations. Also in this test, an FVC manoeuvre was performed 90 s after every dose step.

Borg dyspnoea scores

Dyspnoea was scored with the Borg scale, ranging from 0 to 10 (no to maximal breathlessness) (244), before the first administration and after each administration of AMP or adenosine, as well as at the end of the test.

Data analysis

Reference values for spirometry were obtained from Quanjer *et al.* (245). The PC_{20} (for AMP) and PD_{20} values (for adenosine) of the bronchial challenge tests were determined by log-linear interpolation between the second-to-last and last FEV_1 value (35). Converting the AMP PC_{20} value into the corresponding PD_{20} value for adenosine (following Table 4-1) allowed for a direct comparison of the provocative doses. $FEF_{50\%}$ values at PD_{20} were calculated by interpolating between the last and second-to-last value. Wilcoxon-signed rank tests were performed to test for differences between the nebulised AMP test and dry powder adenosine test, concerning the PD_{20} values, $FEF_{50\%}$ at PD_{20} values, and changes in Borg dyspnea scores.

Table 4-2. Subject demographic characteristics.

Subject No.	Sex	Age (yrs)	Smoking (pack yrs)	FEV_1 (% pred)	FEV_1/FVC (%)	Medications
1	Male	38	Never	107	76	Alvesco 160 µg b.i.d., formoterol 12 µg b.i.d.
2	Female	35	Ex (5.25)	84	74	No medication
3	Male	28	Ex (3)	91	78	Symbicort 400/12 µg b.i.d., Levocetirizine 5 mg q.d.
4	Female	44	Current (20.25)	81	63	Symbicort 400/12 µg prn (twice/week)
5	Female	47	Ex (0.11)	117	72	Salbutamol prn

Table 4-3. Comparison of the responses to AMP and adenosine per individual subject.

	AMP					Adenosine			
Subject No.	PC_{20}	PD_{20} adenosine	$FEF_{50\%}$ at PD_{20} (L/s)	Δ Borg	No. doses	PD_{20}	$FEF_{50\%}$ at PD_{20} (L/s)	Δ Borg	No. doses
1	10.52	0.93	4.47	-	10	1.53	2.70	-	9
2	29.97	2.65	1.85	0.5	11	2.18	1.64	2	10
3	NA	-	-	2	14	62.12	2.41	5	14
4	1.94	0.17	1.43	0.5	7	0.68	1.33	0	8
5	5.16	0.46	1.72	3	9	1.25	1.69	5	9

PD_{20} adenosine: PC_{20} AMP converted into the corresponding PD_{20} adenosine; Δ Borg: change in Borg dyspnoea score (highest concentration AMP/adenosine - baseline); NA: no 20% fall in FEV_1 was obtained.

RESULTS

Subjects

Five asthmatic subjects were included in the study, whose demographic characteristics are shown in Table 4-2. All had a baseline FEV_1 > 80% predicted on both test days. After instruction and practicing, all subjects performed technically satisfactory inhalations. They generated sufficiently high flow rates and inhaled volumes through the adenosine DPI for complete dose release from the blisters and good dispersion of the powder.

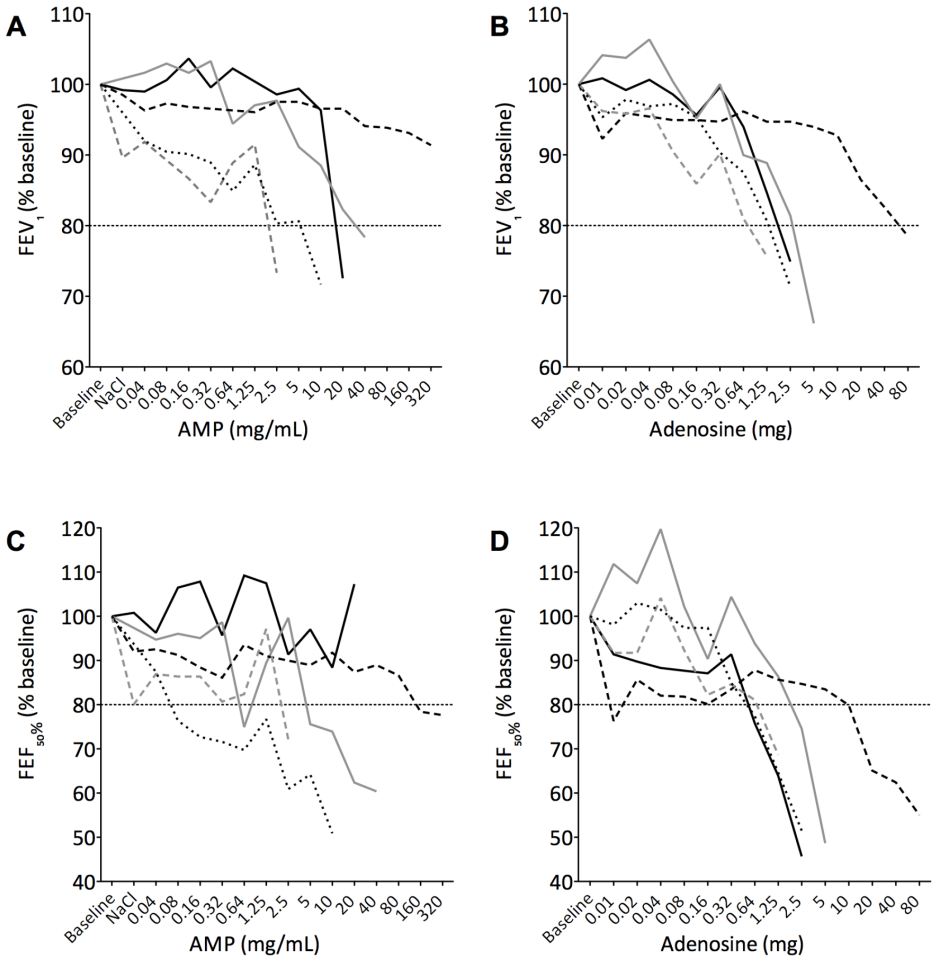

Figure 4-2. Values for FEV_1 (A and B) and $FEF_{50\%}$ (C and D) relative to baseline measured at the consecutive dose steps per individual subject.

Comparison of BHR to adenosine and AMP

All subjects reached a 20% fall in FEV_1 with the new adenosine test (PD_{20}), whereas only four subjects reached this threshold with the AMP test (PC_{20}) (Table 4-3). Subject 3 reached a PD_{20} after inhalation of 80 mg of adenosine, which was two dose steps higher than the highest concentration of AMP according to the calculated dose range for dry powder adenosine. After inhaling 320 mg/mL AMP, the fall in FEV_1 was 9% in this subject.

The numbers of administered doses were comparable for the two tests, as a result of leaving out the two lowest dose steps in the new dry powder test (Table 4-3). PD_{20} AMP and PD_{20} adenosine were not significantly different ($p = 0.144$). In Figure 4-2, the courses of the FEV_1 (A and B) during both tests are given for all individual subjects.

All subjects showed a more than 20% fall in $FEF_{50\%}$ with the dry powder adenosine test compared to four subjects with the AMP test (Figure 4-2, C and D). The $FEF_{50\%}$ at 20% fall of the FEV_1 (Table 4-3, $n = 4$) appeared to be lower after challenge with dry powder adenosine, but this difference did not reach statistical significance ($p = 0.068$).

Safety and subject experience of the dry powder adenosine test

The deepest declines in FEV_1 measured in this study were 28.3% on AMP and 33.8% on adenosine. All subjects recovered to 90% of baseline FEV_1 within 15 min after administration of salbutamol 400 µg. No safety issues and no serious adverse events were encountered during or after the challenges.

The dry powder adenosine was well tolerated by all subjects. The inhalation of dry powder adenosine did not appear to induce more coughing than the inhalation of nebulised AMP. A bitter taste was sometimes reported for the doses that consisted of pure adenosine (> 2.5 mg), whilst at the lower doses, the sweetness of lactose predominated.

Maximum Borg scores reported for the challenges with AMP and adenosine were 3 and 5 respectively. Overall, the scores were not significantly different ($p = 0.144$). The entire dry powder adenosine test (including recovery) could be finished within 60 min, compared to 90 min for the nebulised AMP test. All subjects expressed their preference for the dry powder adenosine test over the nebulised AMP test, mostly because it was faster.

DISCUSSION

In this proof-of-concept study, we investigated the efficacy, acceptance, and safety of a new adenosine bronchial challenge test in a small number of asthmatic subjects.

The results demonstrate that a dry powder system of adenosine is suitable for the assessment of bronchial hyperresponsiveness in asthmatic subjects. Dry powder adenosine induces a response in both the FEV_1 and $FEF_{50\%}$ suggesting bronchoconstriction of both large and small airways. This can be explained by the use of an aerosol with a relatively small particle size in combination with a low inspiratory flow rate, a combination that allows for substantial deposition of the stimulus in the periphery of the lungs and thus for a small-airway response (22). Inevitably, a part of the aerosol is deposited in the upper and central airways too, so a response of these airways is to be expected as well. Further research is needed before we can discriminate between small airways dysfunction and large airways dysfunction based on challenge tests with small and large adenosine particles respectively. The next step is therefore to compare different particle sizes and inhalation flows in a group of asthmatic subjects who are extensively characterised with respect to small and large airways dysfunction.

The provocative doses causing a 20% fall in FEV_1 did not differ significantly for the two tests and were less than two doubling doses apart for each of the four subjects who reached the 20% threshold in both tests. BHR can vary each day due to environmental stimuli and a normal variability includes 1.5 dose step within two days for 90% of the patients (35). The dose range that was calculated for dry powder adenosine thus correlates well with the concentration range of AMP. Moreover, with the new test we were able to administer higher doses than the highest AMP concentration, leading to a response in all subjects, including the AMP-negative subject. Based on these results, we consider further development of the test with the current dose range justified.

In this pilot study, no difficulties were encountered with the inhalation manoeuvre. The medium-high resistance of the inhaler facilitated the subjects in attaining (and retaining) the desired low inspiratory flow rate, as well as in extending their inhalations over several seconds. These long inhalations may have an additional beneficial effect on the peripheral deposition of the aerosol particles. After release and inhalation of the aerosol in the first part of the inspiratory manoeuvre, additional (clean) air is inhaled for further transport of the aerosol towards the more distal regions of the lungs.

Importantly, no adverse events were encountered either. Some coughing was reported in response to dry powder adenosine, but not to a larger extent than to nebulised AMP. The low inspiratory flow rate aids in keeping throat deposition to a minimum. The bitter taste that was experienced especially after inhalation of the 20 mg doses did

not impede continuation of the test.

This small pilot study has some minor drawbacks. Firstly, the timelines of the AMP and adenosine protocol were not completely exchangeable, because tidal breathing took two minutes and the slow IVC manoeuvre approximately 10–20 seconds. Although the shorter duration of the adenosine test was considered a benefit by the participating subjects, it may have implications for the response to the stimulus. Secondly, the IVC manoeuvres and 10-second breath hold may have led to smooth muscle relaxation and bronchoprotection, which is probably not present after two minutes of tidal breathing. This bronchoprotective effect may even be greater after inhaling the highest doses of adenosine, since these dose steps consist of two or four blisters. On the other hand, only one subject reached the highest adenosine dose that required four blisters, but still showed a 20% fall in FEV_1.

In conclusion, this study demonstrates that bronchial challenge testing using inhaled dry powder adenosine is feasible. The new dry powder adenosine test has several improvements compared to the AMP test, most importantly the possibility to administer higher doses of the stimulus and the lower burden on the patient. Further studies will be performed with this test concept to study the influence of particle size and inspiratory flow rate on the airway response to dry powder adenosine.